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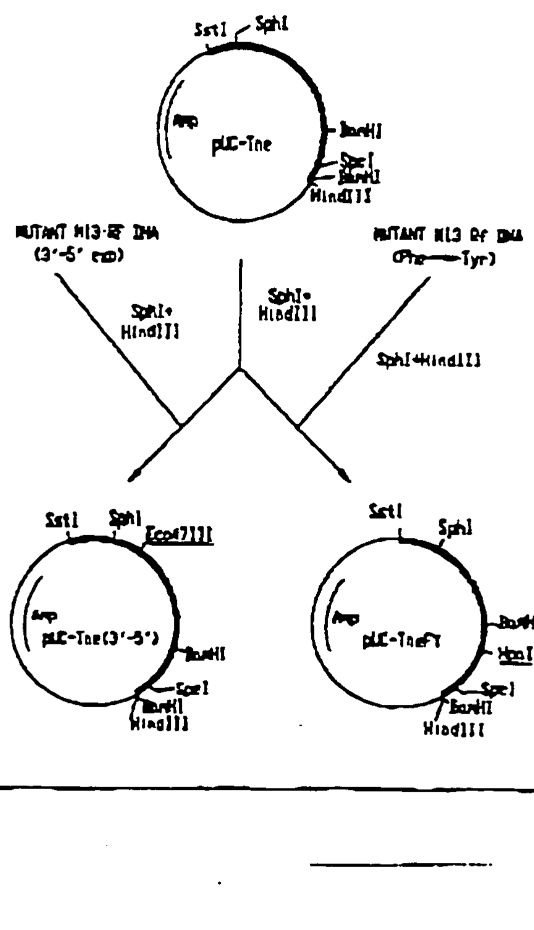
(54) Title: CLONED DNA POLYMERASES FROM THERMOTOGA NEAPOLITANA AND MUTANTS THEREOF

(57) Abstract

The invention relates to a substantially pure thermostable DNA polymerase from *Thermotoga neapolitana* (Tne) and mutants thereof. The Tne DNA polymerase has a molecular weight of about 100 kilodaltons and is more thermostable than Taq DNA polymerase. The mutant Tne DNA polymerase has at least one mutation selected from the group consisting of (1) a first mutation that substantially reduces or eliminates 3' → 5' exonuclease activity of said DNA polymerase; (2) a second mutation that substantially reduces or eliminates 5' → 3' exonuclease activity of said DNA polymerase; (3) a third mutation in the O helix of said DNA polymerase resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides. The present invention also relates to the cloning and expression of the wild type or mutant Tne DNA polymerase in *E. coli*, to DNA molecules containing the cloned gene, and to host cells which express said genes. The Tne DNA polymerase of the invention may be used in well-known DNA sequencing and amplification reactions.



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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: both

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAGCTCACGG GGGATGCAGG AAA 23

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1310 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

ATGGCGAGAC TATTTCTCTT TGATGGCACA GCCCTGGCCT ACAGGGCATA TTACGCCCTC 60

GACAGATCCC TTTCCACATC CACAGGAATT CCAACGAACG CCGTCTATGG CGTTGCCAGG 120

ATGCTCGTTA AATTCATTAA GGAACACATT ATACCCGAAA AGGACTACGC GGCTGTGGCC 180

TTCGACAAGA AGGCAGCGAC GTTCAGACAC AAACTGCTCG TAAGCGACAA GGCGCAAAGG 240

CCAAAGACGC CGGCTCTTCT AGTTCAGCAG CTACCTTACA TCAAGCGGCT GATAGAAGCT 300

CTTGGTTTCA AAGTGCTGGA GCTGGAAGGG TACGAAGCAG ACGATATCAT CGCCACGCTT 360

GCAGCAAAGG GCTGCACGTT TTTTGATGAG ATTTTCATAA TAACCGGTGA CAAGGATATG 420

CTTCAACTTG TAAACGAGAA GATAAAGGTC TGGAGAATCG TCAAGGGGAT ATCGGATCTT 480

GAGCTTTACG ATTCGAAAAA GGTGAAAGAA AGATACGGTG TGGAACCACA TCAGATACCG 540

GATCTTCTAG CACTGACGGG AGACGACATA GACAACATTC CCGGTGTAAC GGGAATAGGT 600

GAAAAGACCG CTGTACAGCT TCTCGGCAAG TATAGAAATC TTGAATACAT TCTGGAGCAT 660

GCCCGTGAAC TCCCCCAGAG AGTGAGAAAG GCTCTCTTGA GAGACAGGGA AGTTGCCATC 720

CTCAGTAAAA AACTTGCAAC TCTGGTGACG AACGCACCTG TTGAAGTGGA CTGGGAAGAG 760

ATGAAATACA GAGGATACGA CAAGAGAAAA CTACTTCCGA TATTGAAAGA ACTGGAGTTT 840

GCTTCCATCA TGAAGGAACT TCAACTGTAC GAAGAAGCAG AACCCACCGG ATACGAAATC 900

GTGAAGGATC ATAAGACCTT CGAAGATCTC ATCGAAAAGC TGAAGGAGGT TCCATCTTTT 960

GCCCTGGACC TTGAAACGTC CTCCTTGGAC CCGTTCAACT GTGAGATAGT CGGCATCTCC 1020

GTGTCGTTCA AACCGAAAAC AGCTTATTAC ATTCCACTTC ATCACAGAAA CGCCCACAAT 1080

CTTGATGAAA CACTGGTGCT GTCGAAGTTG AAAGAGATCC TCGAAGACCC GTCTTCGAAG 1140

ATTGTGGGTC AGAACCTGAA GTACGACTAC AAGGTTCTTA TGGTAAAGGG TATATCGCCA 1200

GTTTATCCGC ATTTTGACAC GATGATAGCT GCATATTTGC TGGAGCCAAA CGAGAAAAAA 1260

TTCAATCTCG AAGATCTGTC TTTGAAATTT CTCGGATACA AAATGACGTC 1310

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 436 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Ala Arg Leu Phe Leu Phe Asp Gly Thr Ala Leu Ala Tyr Arg Ala
1 5 10 15

Tyr Tyr Ala Leu Asp Arg Ser Leu Ser Thr Ser Thr Gly Ile Pro Thr
20 25 30

Asn Ala Val Tyr Gly Val Ala Arg Met Leu Val Lys Phe Ile Lys Glu
35 40 45

His Ile Ile Pro Glu Lys Asp Tyr Ala Ala Val Ala Phe Asp Lys Lys
50 55 60

Ala Ala Thr Phe Arg His Lys Leu Leu Val Ser Asp Lys Ala Gln Arg
65 70 75 80

Pro Lys Thr Pro Ala Leu Leu Val Gln Gln Leu Pro Tyr Ile Lys Arg
85 90 95

Leu Ile Glu Ala Leu Gly Phe Lys Val Leu Glu Leu Glu Gly Tyr Glu
100 105 110

Ala Asp Asp Ile Ile Ala Thr Leu Ala Ala Lys Gly Cys Thr Phe Phe
115 120 125

Asp Glu Ile Phe Ile Ile Thr Gly Asp Lys Asp Met Leu Gln Leu Val
130 135 140

Asn Glu Lys Ile Lys Val Trp Arg Ile Val Lys Gly Ile Ser Asp Leu
145 150 155 160

Glu Leu Tyr Asp Ser Lys Lys Val Lys Glu Arg Tyr Gly Val Glu Pro
165 170 175

His Gln Ile Pro Asp Leu Leu Ala Leu Thr Gly Asp Asp Ile Asp Asn
180 185 190

Ile Pro Gly Val Thr Gly Ile Gly Glu Lys Thr Ala Val Gln Leu Leu
195 200 205

Gly Lys Tyr Arg Asn Leu Glu Tyr Ile Leu Glu His Ala Arg Glu Leu
210 215 220

Pro Gln Arg Val Arg Lys Ala Leu Leu Arg Asp Arg Glu Val Ala Ile
225 230 235 240

Leu Ser Lys Lys Leu Ala Thr Leu Val Thr Asn Ala Pro Val Glu Val
245 250 255

Asp Trp Glu Glu Met Lys Tyr Arg Gly Tyr Asp Lys Arg Lys Leu Leu
260 265 270

Pro Ile Leu Lys Glu Leu Glu Phe Ala Ser Ile Met Lys Glu Leu Gln
275 280 285

Leu Tyr Glu Glu Ala Glu Pro Thr Gly Tyr Glu Ile Val Lys Asp His
290 295 300

Lys Thr Phe Glu Asp Leu Ile Glu Lys Leu Lys Glu Val Pro Ser Phe
305 310 315 320

Ala Leu Asp Leu Glu Thr Ser Ser Leu Asp Pro Phe Asn Cys Glu Ile
325 330 335

Val Gly Ile Ser Val Ser Phe Lys Pro Lys Thr Ala Tyr Tyr Ile Pro
340 345 350

Leu His His Arg Asn Ala His Asn Leu Asp Glu Thr Leu Val Leu Ser
355 360 365

Lys Leu Lys Glu Ile Leu Glu Asp Pro Ser Ser Lys Ile Val Gly Gln
370 375 380

Asn Leu Lys Tyr Asp Tyr Lys Val Leu Met Val Lys Gly Ile Ser Pro
385 390 395 400

Val Tyr Pro His Phe Asp Thr Met Ile Ala Ala Tyr Leu Leu Glu Pro
405 410 415

Asn Glu Lys Lys Phe Asn Leu Glu Asp Leu Ser Leu Lys Phe Leu Gly
420 425 430

Tyr Lys Met Thr
435

What Is Claimed Is:

1. A substantially pure *Thermotoga neapolitana* (Tne) DNA polymerase having a molecular weight of about 100 kilodaltons, or fragments thereof.

2. The DNA polymerase of claim 1, which is isolated from *Thermotoga neapolitana*.

3. The DNA polymerase of claim 2, which is isolated from *Thermotoga neapolitana* DSM 5068.

4. An isolated DNA molecule comprising a gene encoding a Tne DNA polymerase having a molecular weight of about 100 kilodaltons.

5. An isolated DNA molecule of claim 4, wherein the gene is modified to reduce 3'-5' exo activity.

6. The isolated DNA molecule of claim 4, wherein the promoter of said gene is an inducible promoter.

7. The isolated DNA molecule of claim 6, wherein said inducible promoter is selected from the group consisting of a λ-P_L promoter, a *tac* promoter, a *trp* promoter, and a *trc* promoter.

8. A recombinant host comprising a gene encoding Tne DNA polymerase having a molecular weight of 100 kilodaltons.

9. The recombinant host of claim 8, wherein said gene is obtained from *Thermotoga neapolitana*.

10. The recombinant host of claim 9, wherein said gene is obtained from *Thermotoga neapolitana* DSM 5068.

11. The recombinant host of claim 8, wherein said host is prokaryotic.

12. The recombinant host of claim 11, wherein said host is *E. coli*.

13. A method of producing a Tne DNA polymerase having a molecular weight of about 100 kilodaltons, said method comprising:

(a) culturing a cellular host comprising a gene encoding said DNA polymerase;

(b) expressing said gene; and

(c) isolating said DNA polymerase from said host.

14. The method of claim 13, wherein said host is a eukaryotic host.

15. The method of claim 13, wherein said host is a prokaryotic host.

16. The method of claim 15, wherein said prokaryotic host is *E. coli*.

17. A method of synthesizing a double-stranded DNA molecule comprising:

(a) hybridizing a primer to a first DNA molecule; and

(b) incubating said DNA molecule of step (a) in the presence of one or more deoxyribonucleoside triphosphates and Tne DNA polymerase having a molecular weight of about 100 kilodaltons, under conditions sufficient to synthesize a second DNA molecule complementary to all or a portion of said first DNA molecule.

18. The method of claim 17, wherein said DNA polymerase is isolated from *Thermotoga neapolitana*.

19. The method of claim 18, wherein said DNA polymerase is isolated from *Thermotoga neapolitana*, DSM 5068.

20. The method of claim 17, wherein said DNA polymerase is isolated from a recombinant host expressing a gene encoding said DNA polymerase.

21. The method of claim 20, wherein said host is a eukaryotic host.

22. The method of claim 20, wherein said host is a prokaryotic host.

23. The method of claim 22, wherein said prokaryotic host is *E. coli*.

24. The method of claim 17, wherein said deoxyribonucleoside triphosphates are selected from the group consisting of dATP, dCTP, dGTP, dTTP, dITP, 7-deaza-dGTP, dUTP, ddATP, ddCTP, ddGTP, ddITP, ddTTP, [αS]dATP, [αS]dTTP, [αS]dGTP, and [αS]dCTP.

25. The method of claim 24, wherein one or more of said deoxyribonucleoside triphosphates are detectably labeled.

26. The method of claim 25, wherein said detectable label is selected from the group consisting of a radioactive isotope, a fluorescent label, a chemiluminescent label, a bioluminescent label, and an enzyme label.

27. A method of sequencing a DNA molecule, comprising:

(a) hybridizing a primer to a first DNA molecule;

(b) contacting said DNA molecule of step (a) with deoxyribonucleoside triphosphates, Tne DNA polymerase having a molecular weight of about 100 kilodaltons, and a terminator nucleotide;

(c) incubating the mixture of step (b) under conditions sufficient to synthesize a random population of DNA molecules complementary to said first DNA molecule,
wherein said synthesized DNA molecules are shorter in length than said first DNA molecule and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 5' termini; and (d) separating said synthesized DNA molecules by size so that at least a part of the nucleotide sequence of said first DNA molecule can be determined.

28. The method of claim 27, wherein said terminator nucleotide is ddTTP.

29. The method of claim 27, wherein said terminator nucleotide is ddATP.

30. The method of claim 27, wherein said terminator nucleotide is ddGTP.

31. The method of claim 27, wherein said terminator nucleotide is ddCTP.

32. The method of claim 27, wherein said DNA polymerase is isolated from *Thermotoga neapolitana*.

33. The method of claim 32, wherein said DNA polymerase is isolated from *Thermotoga neapolitana* DSM 5068.

34. The method of claim 27, wherein said DNA polymerase is isolated from a recombinant host expressing a gene encoding said DNA polymerase.

35. The method of claim 27, wherein one or more of said deoxyribonucleoside triphosphates is detectably labeled.

36. The method of claim 35, wherein said labeled deoxyribonucleoside triphosphate is [α^{35}S]dATP.

37. A method for amplifying a double stranded DNA molecule, comprising:

(a) providing a first and second primer, wherein said first primer is complementary to a sequence at or near the 3'-termini of the first strand of said DNA molecule and said second primer is complementary to a sequence at or near the 3'-termini of the second strand of said DNA molecule;

(b) hybridizing said first primer to said first strand and said second primer to said second strand in the presence of Tne DNA polymerase having a molecular weight of about 100 kilodaltons, under conditions such that a third DNA molecule complementary to said first strand and a fourth DNA molecule complementary to said second strand are synthesized;

(c) denaturing said first and third strand, and said second and fourth strands with heat; and (d) repeating steps (a) to (c) one or more times.

38. The method of claim 37, wherein said DNA polymerase is isolated from *Thermotoga neapolitana*.

39. The method of claim 38, wherein said DNA polymerase is isolated from *Thermotoga neapolitana* DSM 5068.

40. The method of claim 37, wherein said DNA polymerase is isolated from a recombinant host expressing a gene encoding said DNA Polymerase.

41. A kit for sequencing a DNA molecule, comprising:

(a) a first container means comprising a Tne DNA polymerase having a molecular weight of about 100 kilodaltons;

(b) a second container means comprising one or more dideoxyribonucleoside triphosphates; and

(c) a third container means comprising one or more deoxyribonucleoside triphosphates.

42. The kit of claim 41, wherein said DNA polymerase is isolated from *Thermotoga neapolitana*.

43. The kit of claim 42, wherein said DNA polymerase is isolated from *Thermotoga neapolitana* DSM 5068.

44. The kit of claim 41, wherein said DNA polymerase is isolated from a recombinant host expressing a gene encoding said DNA polymerase.

45. A kit for amplifying a DNA molecule, comprising:

(a) a first container means comprising a Tne DNA polymerase having a molecular weight of about 100 kilodaltons; and

(b) a second container means comprising one or more deoxyribonucleoside triphosphates.

46. The kit of claim 45, wherein said DNA polymerase is isolated from *Thermotoga neapolitana*.

47. The kit of claim 46, wherein said DNA polymerase is isolated from *Thermotoga neapolitana* DSM 5068.

48. The kit of claim 45, wherein said DNA polymerase is isolated from a recombinant host expressing a gene encoding said DNA polymerase.

49. A mutant *Thermotoga neapolitana* DNA polymerase having at least one mutation selected from the group consisting of (1) a first mutation that substantially reduces or eliminates 3'-5' exonuclease activity of said DNA polymerase; (2) a second mutation that substantially reduces or eliminates 5'-3' exonuclease activity of said DNA polymerase; and (3) a third mutation in the O helix of said DNA polymerase resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides, or fragments thereof.

50. The mutant *Thermotoga neapolitana* DNA polymerase as claimed in claim 49, wherein said third mutation is a Phe^{67}-Tyr^{67} substitution.

51. The mutant *Thermotoga neapolitana* DNA polymerase as claimed in claim 49, wherein said first mutant is a Asp^{322}-Ala^{322} substitution.

52. The mutant *Thermotoga neapolitana* DNA polymerase as claimed in claim 49, wherein said mutant polymerase comprises both a Phe^{67} - Tyr^{67} substitution and a Asp^{322} - Ala^{322} substitution.

53. The mutant *Thermotoga neapolitana* DNA polymerase as claimed in claim 49, wherein said mutant polymerase is devoid of the N- terminal 5'- 3' exonuclease domain.

54. The mutant *Thermotoga neapolitana* DNA polymerase as claimed in claim 53, wherein said mutant polymerase is devoid of the 219 N-terminal amino acids of *Thermotoga neapolitana* DNA polymerase.

55. An isolated DNA molecule comprising a DNA sequence encoding a mutant *Thermotoga neapolitana* DNA polymerase having at least one mutation selected from the group consisting of (1) a first mutation that substantially reduces or eliminates 3'-5' exonuclease activity of said DNA polymerase; (2) a second mutation that substantially reduces or eliminates 5'-3' exonuclease activity of said

DNA polymerase; and (3) a third mutation in the O helix of said DNA polymerase resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides, or fragments thereof.

56. The isolated DNA molecule as claimed in claim 55, wherein said DNA molecule is selected from the group consisting of pTrcTne35, pTrcTneFY, pTrcTne35FY, and pTTQTne535FY.

57. The isolated DNA molecule as claimed in claim 55, wherein said DNA molecule further comprises expression control elements.

58. The isolated DNA molecule as claimed in claim 57, wherein said expression control elements comprise an inducible promoter selected from the group consisting of λP_L promoter, a tac promoter, a trp promoter, and a trc promoter.

59. A recombinant host comprising a DNA sequence encoding a mutant *Thermotoga neapolitana* DNA polymerase having at least one mutation selected from the group consisting of (1) a first mutation that substantially reduces or eliminates 3'-5' exonuclease activity of said DNA polymerase; (2) a second mutation that substantially reduces or eliminates 5'-3' exonuclease activity of said DNA polymerase; and (3) a third mutation in the O helix of said DNA polymerase resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides, or fragments thereof.

60. A method of producing a Tne DNA polymerase, said method comprising:

(a) culturing a cellular host comprising a gene encoding a mutant *Thermotoga neapolitana* DNA polymerase having at least one mutation selected from the group consisting of (1) a first mutation that substantially reduces or eliminates 3'-5' exonuclease activity of said DNA polymerase; (2) a second

mutation that substantially reduces or eliminates 5′-3′ exonuclease activity of said DNA polymerase; and (3) a third mutation in the O helix of said DNA polymerase resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides, or fragments of said mutant *Thermotoga neapolitana* DNA polymerase;

(b) expressing said gene; and

(c) isolating said mutant *Thermotoga neapolitana* DNA polymerase from said host.

61. The method of producing a *Thermotoga neapolitana* DNA polymerase as claimed in claim 60, wherein said host is *E. coli*.

62. A method of synthesizing a double-stranded DNA molecule, comprising:

(a) hybridizing a primer to a first DNA molecule; and

(b) incubating said DNA molecule of step (a) in the presence of one or more deoxyribonucleoside triphoshates and a mutant *Thermotoga neapolitana* DNA polymerase under conditions sufficient to synthesize a second DNA molecule complementary to all or a portion of said first DNA molecules; wherein:

said mutant *Thermotoga neapolitana* DNA polymerase has at least one mutation selected from the group consisting of (1) a first mutation that substantially reduces or eliminates 3′-5′ exonuclease activity of said DNA polymerase; (2) a second mutation that substantially reduces or eliminates 5′-3′ exonuclease activity of said DNA polymerase; and (3) a third mutation in the O helix of said DNA polymerase resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides, or fragments thereof.

63. The method of synthesizing a double-stranded DNA molecule as claimed in claim 62, wherein said deoxyribonucleoside triphosphates are selected from the group consisting of: dATP, dCTP, dGTP, dTTP, dITP, 7-deaza-dGTP,

dUTP, ddATP, ddCTP, ddGTP, ddITP, ddTTP, [αS]dATP, [αS]dTTP, [αS]dGTP, and [αS]dCTP.

64. The method of synthesizing a double-stranded DNA molecule as claimed in claim 63, wherein one or more of said deoxyribonucleoside triphosphates are detectably labelled.

65. The method of synthesizing a double-stranded DNA molecule as claimed in claim 64, wherein said label is selected from the group consisting of a radioactive isotape, a fluorescent label, a chemiluminessant label, a bioluminescent label, and an enyzme label.

66. A method of sequencing a DNA molecule, comprising:

(a) hybridizing a primer to a first DNA molecule;

(b) contacting said DNA molecule of step (a) with deoxyribonucleoside triphosphates, a mutant *Thermotoga neapolitana* DNA polymerase, and a terminator nucleotide;

(c) incubating the mixture of step (b) under conditions sufficient to synthesize a random population of DNA molecules complementary to said first DNA molecule;

wherein said synthesized DNA molecules are shorter in length than said first DNA molecule and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 5' termini; and

(d) separating said synthesized DNA molecules by size so that at least a part of the nucleotide sequence of said first DNA molecule can be determined.

wherein said mutant *Thermotoga neapolitana* DNA polymerase has at least one mutation selected from the group consisting of (1) a first mutation that substantially reduces or eliminates 3'-5' exonuclease activity of said DNA polymerase; (2) a second mutation that substantially reduces or eliminates 5'-3' exonuclease activity of said DNA polymerase; and (3) a third mutation in the O

helix of said DNA polymerase resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides, or fragments thereof.

67. The method sequencing a DNA molecule as claimed in claim 66, wherein said terminator nucleotide is selected from the group consisting of ddTTP, ddATP, ddGTP, and ddCTP.

68. A method for amplifying a double stranded DNA molecule, comprising:

(a) providing a first and second primer, wherein said first primer is complemetary to a sequence at or near the 3'-termini of the first strand of said DNA molecule and said second primer is complementary to a sequence at or near the 3'-termini of the second strand of said DNA molecule;

(b) hybridizing said first primer to said first strand and said second primer to said second strand in the presence of a *Thermotoga neapolitana* DNA polymerase, under conditions such that a third DNA molecule complementary to said first strand and a fourth DNA molecule complementary to said second strand are synthesized;

(c) denaturing said first and second strand, and said second and fourth strands with heat; and

(d) repeating steps (a) to (c) one or more times,

wherein:

said mutant *Thermotoga neapolitana* DNA polymerase has at least one mutation selected from the group consisting of (1) a first mutation that substantially reduces or eliminates 3'-5' exonuclease activity of said DNA polymerase; (2) a second mutation that substantially reduces or eliminates 5'-3' exonuclease activity of said DNA polymerase; and (3) a third mutation in the O helix of said DNA polymerase resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides.

69. A kit for sequencing a DNA molecule, comprising:

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(a) a first container means comprising a mutant *Thermotoga neapolitana* DNA polymerase;

(b) a second container means comprising one or more dideoxyribonucleoside triphosphates; and

(c) a third container means comprising one or more deoxyribonucleoside triphosphates,

wherein:

said mutant *Thermotoga neapolitana* DNA polymerase has at least one mutation selected from the group consisting of (1) a first mutation that substantially reduces or eliminates 3'-5' exonuclease activity of said DNA polymerase; (2) a second mutation that substantially reduces or eliminates 5'-3' exonuclease activity of said DNA polymerase; and (3) a third mutation in the O helix of said DNA polymerase resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides.

70. A kit for amplifying a DNA molecule, comprising:

(a) a first container means comprising a mutant *Thermotoga neapolitana* DNA polymerase; and

(b) a second container means comprising one or more deoxyribonucleoside triphosphates,

wherein:

said mutant *Thermotoga neapolitana* DNA polymerase has at least one mutation selected from the group consisting of (1) a first mutation that substantially reduces or eliminates 3'-5' exonuclease activity of said DNA polymerase; (2) a second mutation that substantially reduces or eliminates 5'-3' exonuclease activity of said DNA polymerase; and (3) a third mutation in the O helix of said DNA polymerase resulting in said DNA polymerase becoming non-discriminating against dideoxynucleotides.